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Heavy Metals in Woodlice

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SYNOPSIS

The hepatopancreas of woodlice from sites polluted with heavy metals may contain the highest concentrations of zinc, cadmium, lead and copper recorded in a soft tissue of any terrestrial animal. These elements are contained within two types of intracellular granule. The first type, in the S cells of the hepatopancreas, are spherical granules which always contain copper, sulphur and calcium. In woodlice from contaminated sites, these "copper" granules also contain zinc, cadmium and lead. The second type, in the B cells, are flocculent deposits which always contain iron. In woodlice from contaminated sites, these iron granules also contain zinc and lead.

Some new observations on the uptake of heavy metals by juvenile woodlice are described. The concentrations of zinc, cadmium, lead and copper in newly-emerged *Oniscus asellus* Linnaeus are always very low, even in those r
of mothers which have fed on contaminated leaf litter. However, newly-emerged individuals fed on contaminated litter for six weeks accumulate zinc, cadmium, lead and copper within large numbers of "copper" granules in the S cells of the hepatopancreas. The S cells of juveniles fed on litter from an uncontaminated site for the same length of time contain very few granules in which only copper, sulphur and calcium can be detected.

The possible routes of uptake and loss of metals from the digestive system of woodlice are described. The importance of coprophagy is critically examined and it is concluded that consumption of faeces is probably not necessary for the maintenance of adequate reserves of essential elements within the body. Suggestions are made of the areas which are in most need of further research.

ACCUMULATION OF HEAVY METALS BY WOODLICE

Introduction

Studies on heavy metals in terrestrial isopods began more than 20 years ago with the discovery by Wieser (1961) that the hepatopancreas of *Porcellio scaber* collected from disused mine sites in Cornwall, UK, contained concentrations of copper which were the highest ever recorded in the soft tissues of any animal. Recently, concern about the effects of pollution from smelting and mining operations has prompted research on the dynamics of other heavy metals in woodlice.

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To date, only zinc, cadmium, lead and copper have been studied in detail. In this chapter, the literature on the accumulation of these elements in terrestrial isopods is reviewed and some new observations on the uptake of zinc, cadmium, lead and copper by juvenile specimens of *Oniscus asellus* are described. Factors which may affect the extent to which heavy metals are assimilated from the food, such as the structure and function of the digestive system and coprophagy, are critically examined and suggestions are made of the areas which are in most need of further research.

Concentrations in Whole Animals

The mean concentrations of zinc, cadmium, lead and copper in whole woodlice are positively correlated with the levels in leaf litter in a wide range of contaminated and uncontaminated sites (Hopkin & Martin, 1982a). This has led to the suggestion that terrestrial isopods may be good "biological monitors" of environmental contamination by heavy metals (Coughtrey, Martin & Young, 1977; Martin & Coughtrey, 1982; Wieser, Busch & Büchel, 1976). However, the analysis of digests of whole animals conceals the substantial differences which occur between the concentrations of metals in the different organs of the body.

Distribution within the Body

Studies on the distribution of heavy metals within woodlice have shown that the hepatopancreas is by far the most important storage organ of zinc, cadmium, lead and copper (Coughtrey, Martin & Young, 1977; Hopkin & Martin, 1982a; Martin, Coughtrey, Shales & Little, 1980; Wieser, 1961; Wieser & Wiest, 1968). Indeed, although the hepatopancreas constitutes a mean of only about 5% of the dry weight of the animal, it may contain a mean of more than 75% of the zinc, 95% of the cadmium, 80% of the lead and 85% of the copper in the whole body (Hopkin & Martin, 1982a).

The concentrations of zinc, cadmium, lead and copper in the hepatopancreas of *Oniscus asellus* from contaminated sites can reach 1.2%, 0.4%, 2.5% and 3.4% of the dry weight respectively with no apparent ill effects (Table I). However, when the concentrations of zinc and cadmium in the hepatopancreas are greater than about 1.5% and 0.5% of the dry weight respectively, the woodlice are always moribund and move their limbs only weakly when stimulated (Table I). The hepatopancreas of these animals is grey or white instead of the usual yellow/brown colour and the tubules are deformed. Such animals are occasionally found on the spoil tips of disused zinc mines, or in woodlands contaminated by emissions from a primary zinc–cadmium–lead smelting

TABLE I
Concentrations of heavy metals in the hepatopancreas of individual specimens of Oniscus asellus *from five sites in the UK*[a] *($\mu g\ g^{-1}$ dry weight)*

Site details		Zn	Cd	Pb	Cu	Condition
Shipham	Zn mine	12103	3434	7703	2434	healthy
Shipham	Zn mine	27753	7753	8032	2229	moribund
Hallen	near smelting works	6300	4313	7881	8100	healthy
Hallen	near smelting works	18410	6522	8419	9833	moribund
Charterhouse	Pb mine	3008	2534	25745	6368	healthy
Caradon	Cu mine	2321	782	2143	34116	healthy
Midger	uncontaminated	253	278	274	1385	healthy

[a] For further details of sites see Hopkin & Martin (1982a).

works. However, they are common in populations which have been maintained in the laboratory for several months on leaf litter collected from these sites (Hopkin & Martin, 1982b).

Distribution within the Hepatopancreas

The hepatopancreas of terrestrial isopods consists of four blind-ending tubules (six in *Ligia*) which open into the foregut (Fig. 1). The epithelium is differentiated into two types of cell, the B and S cells (Vernon, Herold & Witkus, 1974), which may be derived from a germinative zone at the tips of the tubules (Donadey & Besse, 1972). The B cells are large and project into the lumen of the tubule. They contain extensive deposits of glycogen and lipid (Alikhan, 1972b; Patanè, 1934; Storch, 1982; Szyfter, 1966) which form the main energy reserves of woodlice. The basement membrane is extensively infolded which increases the surface area in contact with the blood (Clifford & Witkus, 1971). The B cells are thought to be involved in the secretion of digestive enzymes and absorption of food material because when woodlice are starved and fed on diets containing different proportions of fat, carbohydrate and protein, considerable changes take place in their ultrastructural appearance (Storch, 1982, 1984). In contrast, the ultrastructure of the smaller S cells is not affected during these feeding experiments and their main function is thought to be the storage of heavy metals (Prosi, Storch & Janssen, 1983).

Intracellular granules containing heavy metals have been observed in digestive and excretory epithelia of a wide range of invertebrates (Brown, 1982; Icely & Nott, 1980; Simkiss, 1976, 1977). In terrestrial isopods from uncontaminated sites, two main types have been recognized. The first consist of dense spherical accumulations of homogeneous material which contain copper, sulphur and calcium (Hopkin & Martin,

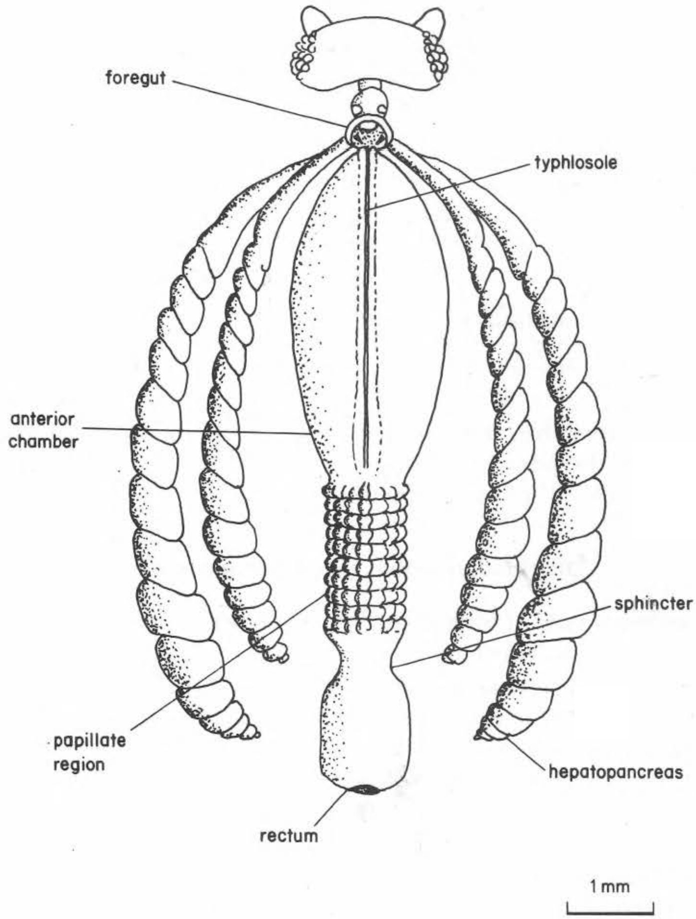


FIG. 1. Schematic diagram of the digestive system of *Oniscus asellus*.

1982b). These "copper" granules occur within the S cells of the hepatopancreas (Alikhan, 1972a; Wieser, 1968; Wieser & Klima, 1969). The second type consist of more loosely-bound deposits of flocculent material which contain iron (Hopkin & Martin, 1982b). These "iron" granules are stored within the B cells of the hepatopancreas (Hryniewiecka-Szyfter, 1972, 1973). However, in woodlice from sites which are contaminated with heavy metals from smelting or mining operations, the "copper" granules may contain zinc, cadmium and lead and the "iron" granules may accumulate zinc and lead (Hopkin & Martin, 1982b). Fine deposits of zinc and lead may also be present on

the membranes of the cells (Hopkin & Martin, 1982b) or scattered throughout the cytoplasm (Prosi *et al.*, 1983).

The "copper" granules of woodlice are insoluble in water, alcohol and acetic acid (Patanè, 1934) and contain about 80% of the total copper in the hepatopancreas (Wieser & Klima, 1969). The results of extensive chemical tests on granules of similar appearance and elemental composition in Amphipoda and Cirripedia have led Icely & Nott (1980) and Walker (1977) to conclude that the copper is associated with an organic compound and is not combined with sulphur as the sulphide. The consistent presence of large amounts of sulphur in the "copper" granules may indicate that the metals are complexed with metallothionein proteins which contain 30% cysteine, a sulphur-containing amino acid. These proteins are ubiquitous in the animal kingdom and have been shown to be of almost identical structure and composition in a wide range of invertebrates and vertebrates (Olafson, Sim & Boto, 1979). Metallothioneins isolated from decapod crustaceans may contain cadmium, copper and zinc (Overnell, 1982; Overnell & Trewhella, 1979; Rainbow & Scott, 1979).

THE UPTAKE OF ZINC, CADMIUM, LEAD AND COPPER BY JUVENILE *ONISCUS ASELLUS*

Introduction

It has been reported that differences in the concentrations of copper between specimens of *Porcellio scaber* from an old mining area, and an uncontaminated site, may persist for several generations in cultures maintained on the same food (Wieser & Makart, 1961). This suggests that genetic differences between populations may at least partially control the extent to which heavy metals are accumulated from the food. This hypothesis has been tested in this study by measuring the net uptake of zinc, cadmium, lead and copper in juvenile *Oniscus asellus* released from the brood pouch of mothers from an uncontaminated site, and from a site which is heavily contaminated with these elements. The ultrastructure of the hepatopancreas of these animals has been studied by light microscopy (LM), transmission and scanning electron microscopy (TEM and SEM) and the elemental composition of intracellular granules determined by X-ray microanalysis.

Materials and Methods

Experimental procedure

Adult *Oniscus asellus* were collected from Wetmoor Wood (British Ordnance Survey Grid Reference ST 743 876) and Haw Wood

(ST 560 798) during May 1982. Wetmoor is an uncontaminated site whereas Haw is 3 km downwind of a primary smelting works and is heavily contaminated with zinc, cadmium, lead and copper (Martin, Duncan & Coughtrey, 1982).

Ten ovigerous females from each population were transferred to individual Petri dishes containing litter from their "own" site and examined daily until they had released their young. Five broods from each group of females were each divided into two and placed in factorial combination in individual Petri dishes containing well-rotted uncontaminated or contaminated leaves of field maple (*Acer campestre*) collected from Wetmoor or Haw respectively (Table II). The other 10 broods and the 20 females were prepared for analysis by atomic absorption spectrophotometry (AAS). The dishes were kept on a moist base within a covered plastic tank for six weeks at a temperature of between 14 and 18 °C. At the end of this period, 10 juveniles were pooled from each dish and prepared for AAS. Rearing woodlice from the same broods on litter from the two sites ensured that any differences in the concentrations of metals in the animals at the end of the experiment which were due to the treatments could be distinguished from those due to genetic differences between and within broods of mothers from the same site.

TABLE II
Concentrations of heavy metals in field maple litter used in the experiments ($\mu g\, g^{-1}$ dry weight)

Site	Zn	Cd	Pb	Cu
Wetmoor	72.1	0.6	37.1	12.8
Haw	807	5.7	502	42.0

Tubules from the hepatopancreas of newly-released young and six-week-old juveniles from all combinations of treatments were prepared for TEM, SEM, LM and X-ray microanalysis.

Preparation of samples

Samples for analysis by AAS were digested in boiling concentrated nitric acid, diluted with de-ionized distilled water and analysed for zinc, cadmium, lead and copper by flame (Varian AA775) or flameless (Varian AA6 and CRA90) methods (for further details see Hopkin & Martin, 1982a).

Tissues for TEM, SEM, LM or X-ray microanalysis were fixed in 2.5% glutaraldehyde and/or 1% osmium tetroxide in 0.1 M cacodylate buffer and dehydrated in a graded series of ethanols. Specimens for TEM, LM and X-ray microanalysis were embedded in Spurr's epoxy resin.

TABLE III

Concentrations of heavy metals within female Oniscus asellus *from which the young used in the experiment were derived (μg g^{-1} dry weight, mean ± standard error,* n = *10).*

Tissue	Dry weight (mg)	Zn	Cd	Pb	Cu
Wetmoor					
hepatopancreas	1.064 ± 0.168	237 ± 48	362 ± 118	340 ± 111	1616 ± 479
gut	1.577 ± 0.316	106 ± 15	2.5 ± 0.4	26.9 ± 5.2	66.4 ± 12.0
rest	17.533 ± 1.324	48.1 ± 2.2	0.61 ± 0.17	1.2 ± 0.1	50.3 ± 4.5
total	20.174 ± 1.574	63.0 ± 4.8	15.7 ± 3.3	16.7 ± 3.6	115 ± 13.2
Haw					
hepatopancreas	1.038 ± 0.081	4649 ± 600	2336 ± 245	6564 ± 1071	6329 ± 741
gut	1.615 ± 0.186	472 ± 104	36.5 ± 2.6	706 ± 88	145 ± 13
rest	15.537 ± 1.444	61.3 ± 8.4	3.9 ± 0.6	18.8 ± 2.2	56.8 ± 2.4
total	18.190 ± 1.639	358 ± 38	138 ± 10	443 ± 52	411 ± 28

Sections of up to 1 μm in thickness were cut on to water, picked up on uncoated aluminium grids and examined in a Philips 400T TEM operating at 80 kV. Areas of the section were analysed with an Edax 90100 energy dispersive X-ray microanalyser. Specimens for SEM were transferred to acetone, frozen and fractured under liquid nitrogen, air dried, mounted on aluminium stubs, coated with gold and examined in a Philips 501B SEM (for further details see Hopkin & Martin, 1982b). Sections of 1 μm in thickness were cut for LM and stained with an aqueous solution of 1% toluidine blue and 1% borax.

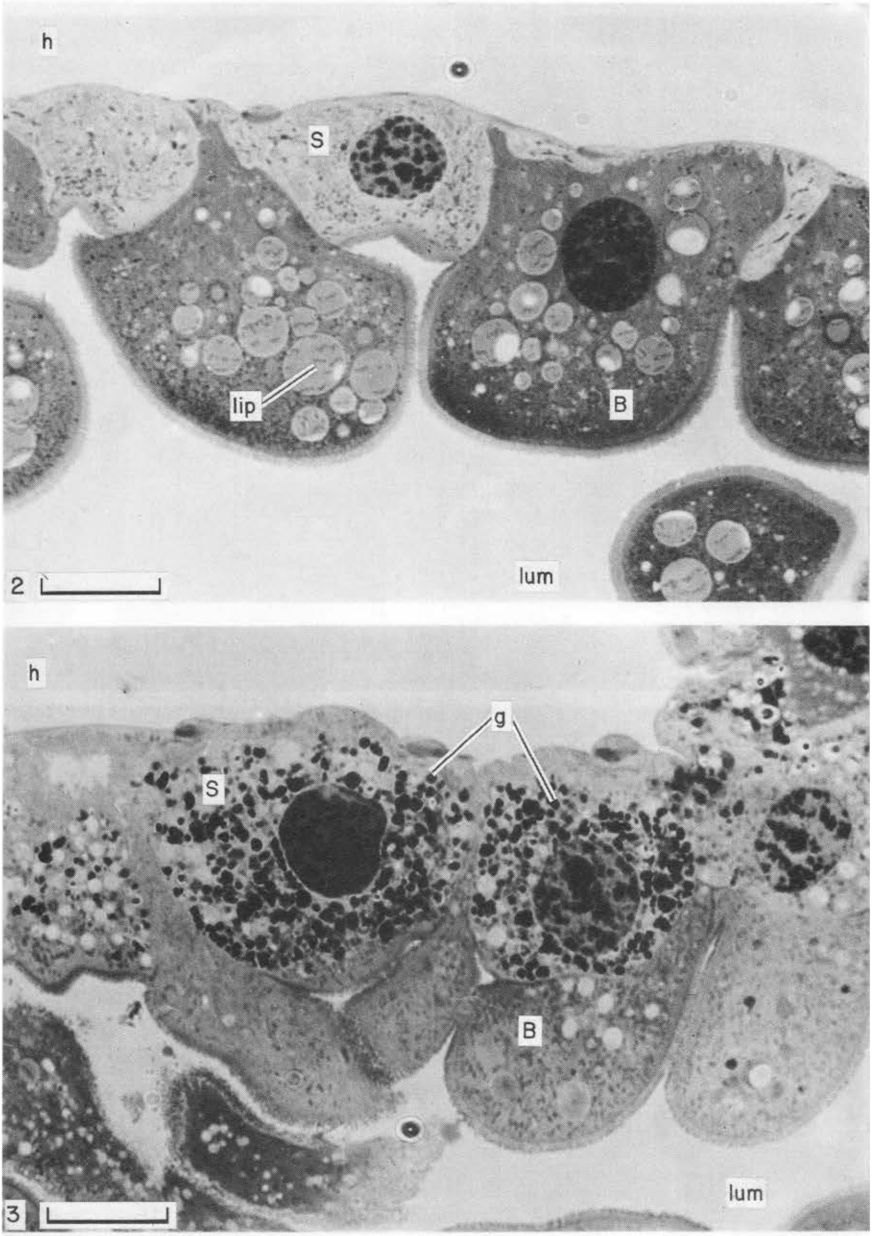
Results and Discussion

Adult female *Oniscus asellus* from Haw contain much higher concentrations of zinc, cadmium, lead and copper than woodlice from Wetmoor (Table III). However, individuals analysed within 24 h of release from the brood pouch contain the same concentrations of these elements, irrespective of the site from which their mothers are derived (Table IV).

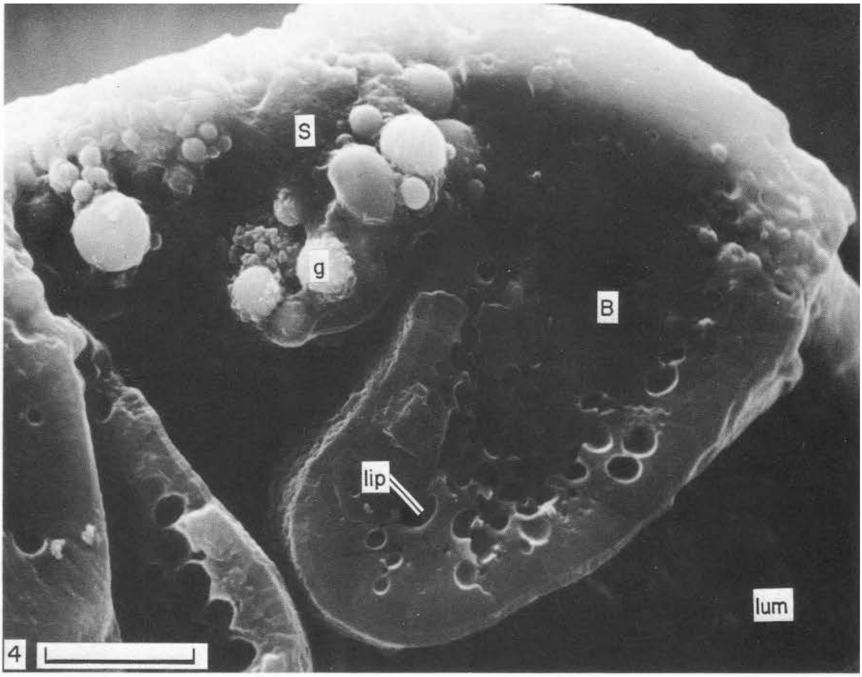
Newly-released individuals reared on field maple leaves from Haw accumulate much greater amounts of zinc, cadmium, lead and copper than juveniles maintained on leaves from Wetmoor (Table V). However, there are no significant differences in the concentrations of these elements between young released by mothers from the two sites if they are fed on the same food. Thus, the suggestion that "genetic factors" may control the extent to which copper accumulates in the tissues (Wieser, 1979) is not supported by these observations.

The hepatopancreas is already differentiated into S and B cells when young woodlice are released from the brood pouch although no "copper" or "iron" granules could be detected in woodlice at this early stage in their development. This confirms the observations of Prosi *et al.* (1983) who could not detect lead in the hepatopancreas of juvenile *Porcellio scaber* derived from mothers which contain very high concentrations of this element. After six weeks of growth, the S cells of juveniles from both sites fed on field maple leaves from Wetmoor contain very small numbers of "copper" granules (Fig. 2) in which copper, sulphur and calcium can be detected by X-ray microanalysis. In contrast, the S cells of juveniles from both sites fed on contaminated leaves from Haw are packed with numerous "copper" granules (Figs 3, 4, 5) which contain zinc, cadmium, lead, copper, sulphur and calcium. "Iron" granules have never been observed and it is assumed that these begin to accumulate in the juveniles at a later stage in their development.

In terrestrial isopods, a convenient measure of the relative extent to which different heavy metals are accumulated is the "concentration factor". This is calculated by dividing the concentration of the metal



FIGS 2, 3. Light micrographs of B cells (B) and S cells (S) within the hepatopancreas of two specimens of *Oniscus asellus*, six weeks after release from the same brood pouch of a female from Wetmoor. The S cells of the juvenile reared on litter contaminated with heavy metals from Haw (Fig. 3) contain far more "copper" granules (g) than the S cells of the juvenile reared on uncontaminated litter from Wetmoor (Fig. 2). h, haemocoel; lip, lipid; lum, lumen of hepatopancreas tubule. Scale bars = 20 μm.



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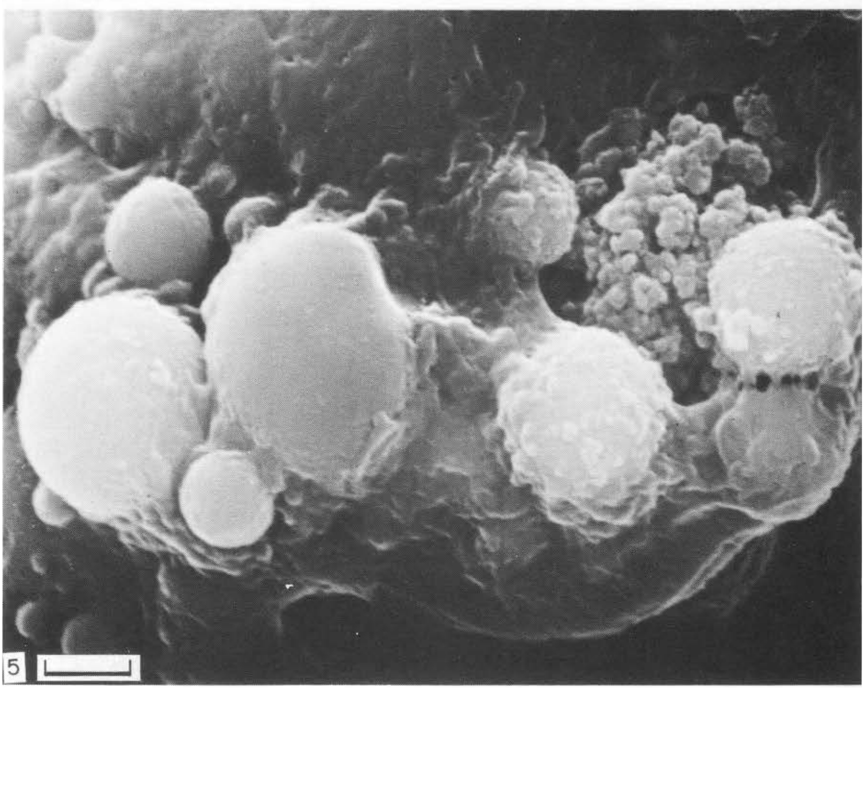

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TABLE IV

Concentrations of heavy metals in young Oniscus asellus *within 24 h of release from the brood pouch (μg g^{-1} dry weight, means of five broods from mothers from each site)*

	Number in brood	Total dry weight (mg)	Zn	Cd	Pb	Cu
Wetmoor						
mean	46	6.6	8	<0.25	<1.0	69
range	(33–58)	(4.5–8.4)	(<1–12)			(54–100)
Haw						
mean	39	6.0	7	<0.25	<1.0	64
range	(25–53)	(3.8–7.9)	(2–11)			(43–121)

TABLE V.

Concentrations of heavy metals and concentration factors (c.f.) in juvenile Oniscus asellus *after feeding for six weeks on field maple litter (μg g^{-1} dry weight, means of five groups of pooled samples of 10 woodlice)*

	Dry weight (mg)	Zn	Cd	Pb	Cu
Wetmoor woodlice/Wetmoor litter					
mean	12.0	27	3.4	6.2	85
range	(9.2–14.1)	(18–42)	(1.0–12)	(<1–13)	(63–99)
c.f.		0.37	5.7	0.17	6.6
Haw woodlice/Wetmoor litter					
mean	11.5	31	4.7	5.0	95
range	(8.3–13.5)	(22–49)	(1.5–10)	(<1–15)	(60–121)
c.f.		0.43	7.8	0.13	7.4
Wetmoor woodlice/Haw litter					
mean	10.2	124	22	38	294
range	(9.2–11.0)	(50–136)	(15–47)	(25–49)	(153–410)
c.f.		0.15	3.9	0.07	7.0
Haw woodlice/Haw litter					
mean	10.5	106	28	48	256
range	(8.9–12.0)	(74–111)	(18–42)	(30–81)	(127–371)
c.f.		0.13	4.9	0.10	6.1

FIGS 4, 5. Scanning electron micrographs of a transversely fractured tubule from the hepatopancreas of a juvenile *Oniscus asellus* from a female from Wetmoor. This individual was reared for six weeks on litter contaminated with heavy metals from Haw. The S cells (S) are packed with large ''copper'' granules (g) which are shown in greater detail in Fig. 5, B, B cell; lip, space left by lipid droplets dissolved out during preparation; lum, lumen of hepatopancreas tubule. Scale bars = 10μm and 2μm respectively.

in the body by the concentration in leaf litter on which the animal has been feeding. In general, concentration factors for zinc and lead in whole woodlice are less than one, whereas for cadmium and copper they are about five or more (Hopkin & Martin, 1982a; Hunter & Johnson, 1982; Joosse, Wulffraat & Glas, 1981; Martin & Coughtrey, 1976, 1981; Martin, Coughtrey & Young, 1976; Wieser, 1979; Wieser, Dallinger & Busch, 1977; Williamson, 1979). The concentration factors for these metals in *Oniscus asellus* reared on field maple leaves for six weeks are in good agreement with these findings (Table V). Some of the possible reasons why cadmium and copper are assimilated much more readily than zinc or lead are examined in the next section.

FACTORS INFLUENCING THE EXTENT TO WHICH HEAVY METALS ARE ACCUMULATED BY WOODLICE

Structure and Function of the Digestive System

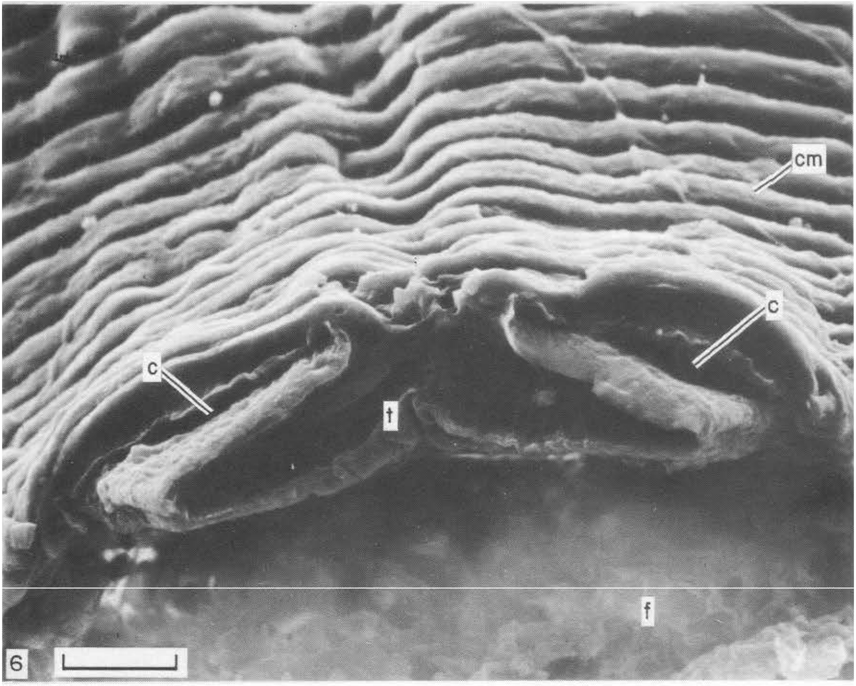
The only significant route by which heavy metals can be assimilated or excreted in terrestrial isopods is *via* the digestive system. Therefore, in order to understand why much greater proportions of cadmium and copper than of zinc or lead are assimilated from food, it is essential to examine the structure and function of the digestive organs. Much of the description which follows is based on the studies on *Philoscia muscorum* by Hassall (1977) and Hassall & Jennings (1975).

The hindgut consists, simply, of a straight tube between the foregut and the anus (Fig. 1). It is ectodermal in origin and is lined with cuticle throughout its whole length (Holdich, 1973). There is no peritrophic membrane so food in the lumen is in direct contact with the cuticular surface (Hartenstein, 1964; Holdich & Mayes, 1975; see also Fig. 7). The tubules of the hepatopancreas are endodermal in origin and are not lined with cuticle.

When macerated food enters the foregut, liquids are separated in the proventriculus and pass into the lumen of the hepatopancreas where heavy metals in solution can be absorbed by the S and B cells and bound within "copper" or "iron" granules. Solids pass into the anterior

FIG. 6. Scanning electron micrograph of a transverse fracture through the anterior chamber of the hindgut of *Oniscus asellus* showing the typhlosole (t). The dorsal infolding forms two parallel channels (c) which carry digestive enzymes from the hepatopancreas to the papillate region. cm, circular muscles; f, food material in the lumen. Scale bar = 20 μm.

FIG. 7. Light micrograph of a transverse section through the anterior chamber of the gut. There is no peritrophic membrane so food (f) in the lumen is in direct contact with the cuticular surface (cu) of the epithelial cells. h, haemocoel. Bar = 20 μm.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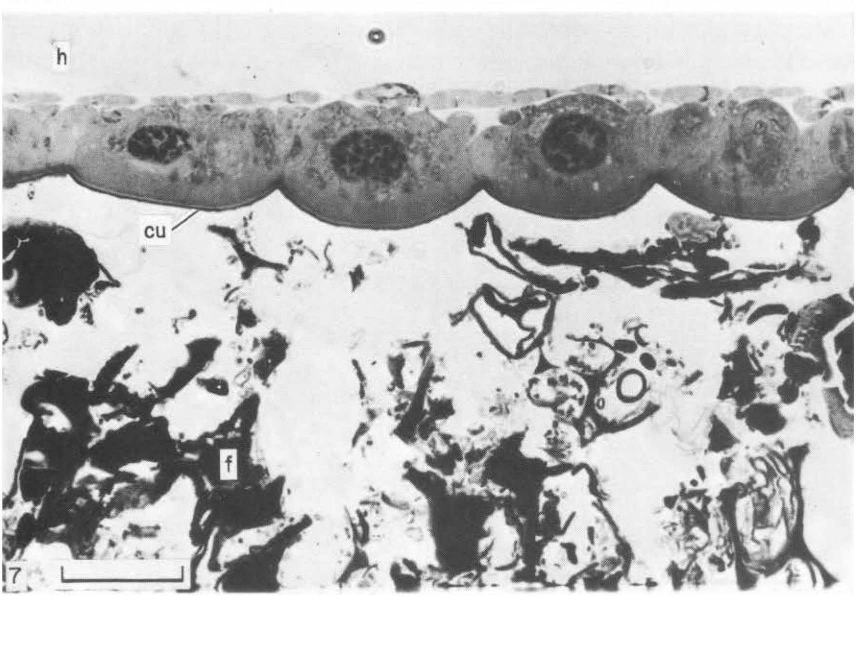
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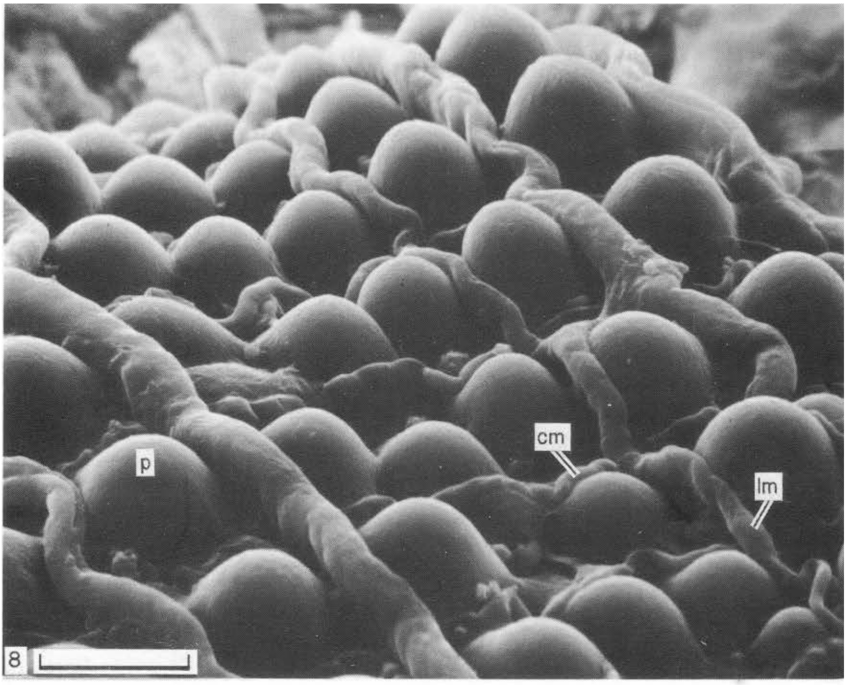

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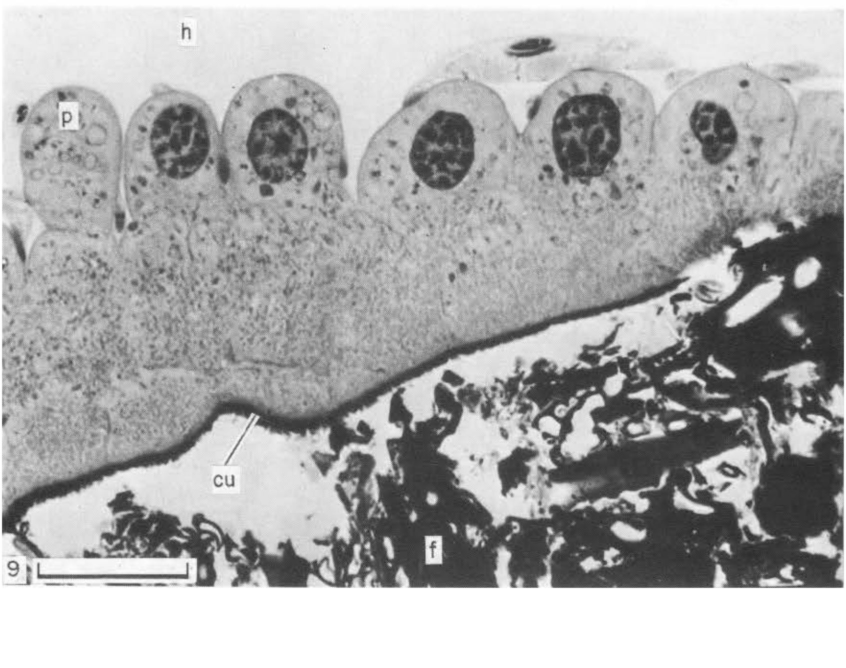

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chamber of the hindgut (Fig. 1) where the activity of micro-organisms within the leaf fragments increases, particularly with regard to the production of cellulases which the animals do not manufacture for themselves (Hassall & Jennings, 1975). This chamber stores a large amount of food which is passed posteriorly into the papillate region (Fig. 1) as space is created by the loss of faecal material.

The anterior chamber of the hindgut has a dorsal infolding or typhlosole (Fig. 1). The lateral extensions of this structure form two parallel channels beneath the roof of the hindgut (Fig. 6). When the anterior chamber is full, the woodlouse ceases to feed and the hepatopancreas secretes digestive enzymes (Hartenstein, 1964) which are carried via the typhlosole to the papillate region (Murlin, 1902). In the papillate region, heavy metals released by microbial activity in the anterior chamber, and by digestive enzymes, can be absorbed by the epithelial cells. The transfer of metals into the blood may be facilitated by the large surface area created by the basal projections of the cells (Figs 8 and 9).

The papillate region is separated from the rectum by a powerful sphincter. When this relaxes, residual matter is forced into the rectum where it is compacted into faecal pellets and voided.

Thus, the amounts of zinc, cadmium, lead and copper which are assimilated from the food will depend first on the amounts of each metal which are carried in the liquid fraction into the lumen of the hepatopancreas from the proventriculus, secondly, on the amounts which are released into solution by enzymes in the lumen of the digestive system and thirdly, on the relative efficiencies of the mechanisms for the transport of each element into the cells of the hepatopancreas and the papillate region.

"Availability" in the Food

If the differences between the concentration factors were due solely to differences in the "availability" of metals in the food, then the amounts of zinc and lead released into solution in the digestive system would be less than one tenth of the amounts of cadmium and copper (Table V). However, experiments in which the "availability" of these elements has been assessed in leaf litter have shown that about 50% of the zinc,

FIG. 8. Scanning electron micrograph of the outside of the papillate region of the hindgut. The basal regions of the cells (p) project into the haemocoel between the circular (cm) and longitudinal (lm) muscles. Bar = 20 μm.

FIG. 9. Light micrograph of an oblique section through the papillate region of the hindgut. The basal projections of the cells (p) increase the surface area in contact with the blood. cu, cuticular surface of the epithelial cells; f, food material in the lumen; h, haemocoel. Bar = 20 μm.

cadmium and copper are soluble in chemical extractants chosen to simulate the action of digestive enzymes, but that very much smaller amounts of lead are released (Martin, Coughtrey & Young, 1976). Thus, differences in "availability" could account for the small concentration factors for lead, but differences between the assimilation rates for zinc, and cadmium and copper, must be due to a lower efficiency of uptake of zinc by the hepatopancreas and papillate region of the hindgut.

Relative Efficiency of Uptake from the Food

Terrestrial isopods must have evolved efficient ways of assimilating essential elements from the food because, unlike their marine ancestors, they can no longer obtain them directly from the external medium across the respiratory surfaces (Edney, 1968; Wieser, 1968). Ideally, the uptake of essential heavy metals would be controlled by feedback mechanisms which responded to deficiencies or excesses of these elements in the tissues. However, it is doubtful whether such mechanisms exist in terrestrial isopods and if they do, whether they operate with a high degree of efficiency (Wieser, 1979).

Copper is present in very low concentrations in leaf litter: about 10 to 20 μg^{-1} dry weight (Guha & Mitchell, 1966; Hopkin & Martin, 1982a). Since it is required as an essential part of the oxygen-carrying blood protein haemocyanin (Bonaventura & Bonaventura, 1980), assimilation from the lumen of the digestive system must be very efficient. Indeed, more than 90% of the copper in the food is assimilated by *Porcellio laevis* over a 14-day period if the animals are fed on leaves enriched with a soluble salt of the metal (Dallinger & Wieser, 1977). Zinc, in contrast, is present in much higher concentrations in uncontaminated leaf litter (about 50 to 300 $\mu g\, g^{-1}$ dry weight, Guha & Mitchell, 1966; Hopkin & Martin, 1982a) so even if the requirement for this element was as high as for copper, the mechanisms for its uptake would not need to be so efficient.

The evolution of a highly efficient system for the assimilation of copper may have two disadvantages. First, there are likely to be occasions when more copper is assimilated than is required to satisfy the immediate physiological needs of the animal. Secondly, cadmium may be taken up along the same biochemical pathway since it has been shown to bind to copper metallothionein in other Crustacea (Overnell & Trewhella, 1979). The small proportion of lead accumulated from the food may follow the same pathways as calcium (Beeby, 1978). The possible ways in which woodlice may have solved these problems are examined in the next section.

Storage within the Hepatopancreas

The recent suggestion by Simkiss (1983) that zinc, cadmium and copper may pass across cell membranes more than a million times faster than sodium or potassium implies that non-essential elements, and essential heavy metals which are surplus to requirements, must be rapidly excreted, or stored in an insoluble form to prevent them from diffusing throughout the body and interfering with biochemical reactions within the tissues. To adopt the first solution to this problem would involve the expenditure of considerable amounts of energy in maintaining concentration gradients between the cells of digestive organs and the digestive fluids (see Simkiss, 1976, 1977, for a more detailed discussion of this concept). The presence of "copper" and "iron" granules within the hepatopancreas suggests that woodlice may have adopted the second solution and may regulate the concentrations of heavy metals in the blood by controlling the amounts which are precipitated on to these deposits. Thus, the primary role of granules within the hepatopancreas is probably the "detoxification" of heavy metals and not the storage of essential elements suggested by Hopkin & Martin (1982b). Small reserves of loosely-bound essential elements could be maintained to supply biochemical processes when demand from the tissues exceeded that which could be supplied from the food.

The concentrations of cadmium and copper in *Oniscus asellus* from Haw do not decrease when they are maintained on leaf litter from Wetmoor for five months (Hopkin & Martin, in prep.) This suggests that once these metals are deposited within "copper" or "iron" granules, they are retained within the animal until it dies. Indeed, even if all the cadmium and copper was extracted from the food as it passed through the digestive system, a one-year-old woodlouse from Wetmoor or Haw weighing 20 mg (dry weight) would have to have eaten about 5% of its weight in leaf litter per day to account for the total amounts of these metals contained within the body. This rate of feeding is well within the range of 1% to 10% of body weight per day which has been measured in the laboratory (Wieser, 1979).

In uncontaminated or moderately contaminated sites, the rate of accumulation of heavy metals is directly proportional to the rate of growth and the concentrations within the body do not increase with age. However, at sites which are heavily contaminated, the rate of accumulation exceeds the rate of growth (Fig. 10). The amount of metal within the animal increases exponentially (Fig. 10) until the capacity of the hepatopancreas to "detoxify" heavy metals is exceeded, the concentrations in the blood increase to toxic levels and the animals are killed. The observation reported on pp. 144–145 indicates that there are

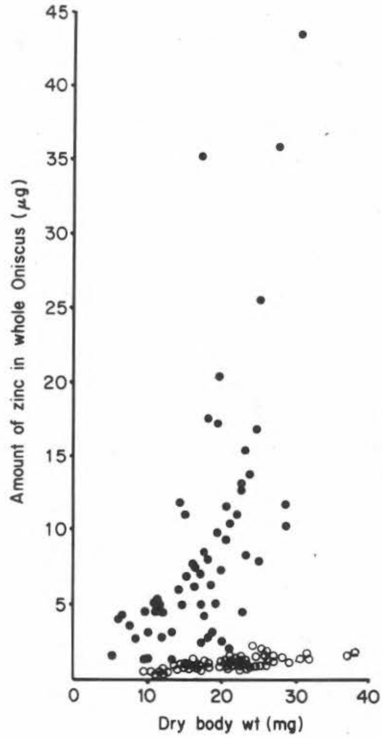

FIG. 10. Plot of total content of zinc against dry body weight of individual *Oniscus asellus* from Shipham (zinc mine, solid circles) and Midger Wood (uncontaminated site, open circles). The three woodlice with more than 35 μg of zinc in the body were moribund.

at least two areas in the UK where *Oniscus asellus* are being affected in such a way, almost certainly by an excessive intake of zinc and cadmium.

Coprophagy

The relationship between coprophagy and copper balance has been studied in more detail than any other topic concerned with heavy metals in terrestrial isopods. Workers in this field can be split into two groups. The first group believe that, under certain circumstances, woodlice cannot extract adequate amounts of copper from leaf litter and have to resort to coprophagy to obtain the metal in a form which is more easily assimilated (Dallinger & Wieser, 1977; Debry & Lebrun, 1979; Wieser, 1965a, 1966, 1967, 1968, 1978, 1979; Wieser, Dallinger *et al.*, 1977). The second group believe that adequate amounts of copper can be obtained without eating faeces (Coughtrey, Martin, Chard & Shales, 1980; Hassall & Rushton, 1982; White, 1968). Both

groups have presented experimental evidence which supports their claims.

This confusion is probably due to differences in the experimental procedures employed by the two groups and, in particular, the methods by which the "availability" of copper in the food and faeces has been assessed.

When a woodlouse takes in food, it is broken down into tiny pieces, the contents of cells are released and a much greater surface area is exposed to attack by digestive enzymes. Thus, subjecting large fragments of leaf material to the action of extractants may underestimate the amounts of copper which are released into solution in the gut.

The extractants used may not be reasonable analogues of the digestive enzymes. For example, Dallinger & Wieser (1977) assessed the "availability" of copper in leaf litter and faeces by extraction with 0.1 N hydrochloric acid which has a pH of 1.1. This is clearly an inappropriate substance with which to attempt to mimic the action of digestive enzymes which have a pH of between 6.2 and 6.8 (Hartenstein, 1964).

Several researchers have performed experiments designed to measure the balance between assimilation and excretion of zinc (Joosse *et al.*, 1981), lead (Beeby, 1978) and copper (Dallinger & Wieser, 1977; Debry & Lebrun, 1979) using leaf litter which has been enriched in the laboratory with simple salts of individual metals. This type of experiment may be unrealistic because woodlice are unlikely to encounter metals in this form in the wild.

Experiments in which woodlice have been shown to be capable of "choosing" litter which contains preferred concentrations of copper (Dallinger, 1977) or zinc (Joosse *et al.*, 1981) should also be treated with caution. The animals are probably responding to differences in the "taste", texture, extent of microbial decay and concentrations of anions such as sulphate, rather than to differences in the levels of metals.

A recent paper by Hassall & Rushton (1982) has demonstrated conclusively that coprophagy is not essential for the growth of woodlice and that copper is not a critically limiting nutrient in terrestrial isopods. These authors measured the relative amounts of litter and faeces eaten, and the growth of *Porcellio scaber* fed on leaves of *Betula pendula* containing different concentrations of copper. Their results can be summarized as follows:

(a) Isopods on litter containing naturally low levels of copper (33.7 $\mu g\ g^{-1}$ dry weight, 5.6 $\mu g\ g^{-1}$ EDTA extractable) and allowed to ingest faeces from an established culture (13.8 $\mu g\ g^{-1}$ dry weight, 8.8 $\mu g\ g^{-1}$ EDTA extractable) increased in weight by 33% over 50 days whereas those maintained on the same food but prevented from eating faeces increased in weight by only 25% in the same period.

However, woodlice allowed access to faeces also grew better than those prevented from indulging in coprophagy when the experiment was repeated using litter from spoil tips of copper mines which contained high levels of copper (124 $\mu g\, g^{-1}$ dry weight, 62.1 $\mu g\, g^{-1}$ EDTA extractable).

(b) Woodlice fed on leaves enriched with copper sulphate (282 $\mu g\, g^{-1}$ dry weight, 111 $\mu g\, g^{-1}$ EDTA extractable) increased in weight by only 22%, less than the increase of 25% achieved by woodlice deprived of their faeces on the natural "low copper" diet.

(c) When the "low copper" diet was supplemented with shredded carrot (5.4 $\mu g\, g^{-1}$ dry weight of copper), woodlice deprived of faeces, and those able to indulge in coprophagy, both grew by more than 50% over 50 days. Thus carrot and faeces contain nutrients, other than copper, in forms which are more "available" to woodlice than they are in leaf litter.

(d) Faeces formed a greater proportion of the diet when the animals were fed on freshly fallen rather than decayed litter.

Hassall & Rushton (1982) have suggested that enhanced microbial activity increases the nutrient status of the faeces in such a way that some coprophagy is "necessary" for woodlice to optimise their overall nutrient uptake. However, in the wild, coprophagy probably occurs very rarely because of the difficulty of locating faecal pellets among leaf litter once they have been voided. In the laboratory, woodlice probably eat their faeces in preference to freshly fallen litter, not because they can detect that the nutrients within it are more "available" but because there is a strong selective pressure for them to choose food which is moist and decaying. In the wild, such material is almost always of a better nutritional status.

CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

Woodlice are ideal animals in which to study the dynamics of heavy metals. They are easy to collect and maintain in the laboratory and are able to accumulate very high concentrations of these elements in their tissues. However, when laboratory experiments are performed, it is essential that animals are not placed in situations which they could not reasonably be expected to encounter in the field.

Some of the areas which are in most need of further research are outlined below:

(a) What is the rate of turnover of cells in the hepatopancreas? Once "copper" and "iron" granules are formed, do they remain in the animal until it dies?

(b) What are the relative amounts of zinc, cadmium, lead and copper which are released from the food into solution in the digestive system? Once made soluble, what proportions of these elements are taken up by the epithelial cells of the hepatopancreas, and by the papillate region of the hindgut?

(c) Have woodlice in contaminated sites evolved to be more tolerant of high levels of heavy metals in the diet in the same way as freshwater isopods (Brown, 1976, 1977, 1978; Fraser, 1980; Fraser, Parkin & Verspoor, 1978)?

(d) What happens to "copper" and "iron" granules when woodlice moult? Wieser (1965b, 1968) has suggested that the "copper" granules are all dissolved. However, there are no significant differences between the levels of heavy metals in the hepatopancreas of intermoult woodlice and those which are casting their exoskeleton (Alikhan, 1972a; Hopkin & Martin, 1982a).

(e) Finally, and indirectly, what effects do the very high concentrations of zinc, cadmium, lead and copper within adult woodlice in contaminated sites have on the wide range of vertebrates and invertebrates which prey on them (Sunderland & Sutton, 1980; Sutton, 1980)?

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